



Association of absolute lymphocyte and cd4 T cell count ratio with outcome of HIV-negative TB meningitis patients- A longitudinal study.

Dr. Pratyush Kumar^{1*}, Dr. (Prof) Deepak Goel², Dr. Manish Mittal³, Dr. Ashwani Bhatt⁴

Senior Resident, Dept of Neurology, Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India¹

HOD, Dept of Neurology, Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India²

Professor, Dept of Neurology, Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India³

Associate Professor, Dept of Neurology, Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India⁴

Abstract

Background

Tuberculous meningitis (TBM), the most severe form of tuberculosis, presents significant diagnostic and therapeutic challenges. Cellular immunity, particularly CD4+ T lymphocytes and absolute lymphocyte count (ALC), plays a critical role in host defense against *Mycobacterium tuberculosis*. However, the prognostic significance of ALC and CD4 counts in HIV-negative TBM patients remains underexplored in India.

Objective: To assess the association between absolute lymphocyte count and CD4 T cell count with clinical outcomes in HIV-negative TBM patients.

Methods

This was a longitudinal follow-up study conducted in the Department of Neurology, Himalayan Institute of Medical Sciences, Dehradun, over 2 years. A total of 53 HIV-negative adult TBM patients were enrolled and classified by TBM stage (I–III) and diagnostic certainty (definite, probable, possible) using MRC grading and Marais criteria, respectively.

Results

Among 53 patients, 23 were male and 30 were female. Females had significantly better outcomes (83.3% vs 47.8%, $p = 0.006$, RR = 2.36). Patients in earlier TBM stages had higher good outcomes: Stage I (85.7%), Stage II (78.7%), Stage III (30.7%) ($p = 0.004$). No statistically significant association was found between outcome and ALC ($p = 0.424$) or CD4 count group ($p = 0.856$), though trends favored better prognosis in patients with normal immune parameters. Duration of dexamethasone therapy and symptom onset also showed no significant impact on outcome.

Conclusion

While TBM stage and gender were significantly associated with clinical outcome, no definitive link was observed between ALC or CD4 count and prognosis in HIV-negative TBM patients. Further large-scale studies are warranted to validate the prognostic utility of immune markers in TBM.

Recommendations

To confirm the predictive significance of ALC and CD4 counts in HIV-negative TBM, future research should involve larger, multicenter cohorts.

Keywords: Tuberculous meningitis, CD4 count, Absolute lymphocyte count, HIV-negative, Outcome, Neurology, India

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Corresponding author: Dr Pratyush Kumar*

Email: pratyushdeepu@gmail.com

Senior Resident, Dept of Neurology, Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India

Introduction

Tuberculosis (TB) is a very common human infection worldwide, which is caused by *Mycobacterium*

tuberculosis (MTB). Almost one-third of the world's population is infected with latent tuberculosis. Although these people are clinically unaffected, they have



a 10% lifetime risk of developing active disease. Southeast Asia, the Western Pacific, and Africa account for the largest share of the global burden of tuberculosis. In 2020, 30 countries with a high TB burden accounted for 86% of new TB cases. Eight countries account for two-thirds of the total, with India leading the way, followed by China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa. Tuberculous meningitis (TM), the most lethal form of tuberculosis (TB), poses major diagnostic and treatment challenges to healthcare providers worldwide. Patients with TBM present with typical meningitis symptoms and signs, including headache, fever, and neck stiffness. In the early stages, meningeal symptoms may not appear. The duration of symptoms before onset can vary from a few days to several months. TBM cases may present at advanced clinical stages with a Glasgow Coma Score of 10 or less, especially in resource-limited settings. Cranial nerve paralysis (CN), hemiplegia, paraplegia, and seizures are common and may increase the likelihood of developing MTB as a cause of meningitis. Tuberculosis is the prototype of infections that require a cellular immune response for their control. CD8⁺ T-lymphocytes are also important for an effective T-cell immune response.

The host's immune system's capability mostly determines how quickly a TB infection will be eradicated, go into dormancy, or fail, leading to active disease. Protective immunity to TB in humans relies upon both CD4 and CD8 T-cells through cell-mediated responses, allowing full eradication or control of infection.

CD4 T-cells play a significant role in HIV infection, which increases the risk of TB reactivation in infected people due to the depletion of these cell subsets. It has been documented previously that TB infection itself may cause a CD4 lymphopenia in patients not infected with HIV, sometimes with grave consequences. It had also been demonstrated that the CD4 lymphopenia was potentially reversible with treatment.

Davoudi et al in their study, CD4⁺ cell counts in patients with different clinical manifestations of tuberculosis, concluded that patients with lower CD4 counts had more severe disease and poorer condition. [1]

AK Al Aska, in their study CD4⁺ T-lymphopenia in HIV negative tuberculous patients at King Khalid University Hospital in Riyadh, Saudi Arabia, found significantly lower CD4 and CD8 counts among infected HIV negative patients as compared with controls. The post-treatment CD4 counts demonstrated the tendency towards recovery to normal values. Patients with disseminated disease had much lower CD4 values than localized forms, with a delay in returning towards normality. [2]

Uppal et al in their study, Comparison of CD4 and CD8 lymphocyte counts in HIV-negative pulmonary TB patients with those in normal blood donors and the effect of antitubercular treatment, concluded that TB is a reversible cause of CD4 lymphocytopenia and is associated with normal numbers of CD8 cells. The radiologic extent of disease does not seem to determine the immune response [3]

To the best of our knowledge, no prior studies from India have investigated the association between absolute lymphocyte count and CD4 T cell values with clinical outcomes in HIV-negative tuberculous meningitis patients, making the study a novel contribution to the existing literature.

The study was conducted to assess the association between absolute lymphocyte count and CD4 T cell count with clinical outcomes in HIV-negative TBM patients.

Methodology

Study design

A prospective, longitudinal, follow-up study.

Study settings

The study was conducted at the Neurology department of the Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India. This was a longitudinal follow-up study. The study area comprised the Department of Neurology at a tertiary care centre in Uttarakhand. The study was conducted between and, for a total duration of 2 years.

Study population

The study population comprised patients who were admitted to the institute from the Emergency and OPD. After obtaining informed consent from the caregivers and families of the patients, the patients were included in the study according to the following criteria. The inclusion criteria for this study encompassed adult patients aged 18 years and above who were admitted to the Neurology



ward of the Himalayan Institute of Medical Sciences with a clinical diagnosis of tuberculous meningitis. Diagnosis was established based on the Marais criteria and included patients categorized as having definite, probable, or possible TBM. Only those patients who were confirmed to be HIV-negative and who provided informed consent for participation and longitudinal follow-up were included in the study.

The exclusion criteria for this study included patients with confirmed HIV infection, underlying malignancies, those receiving immunosuppressive therapy, and individuals with chronic comorbidities such as chronic kidney disease, chronic liver disease, or rheumatologic disorders. Additionally, patients younger than 18 years of age and those unwilling or unable to provide informed consent were excluded.

Data collection

Data were collected prospectively from adult HIV-negative TBM patients admitted to the neurology department. Diagnosis was made using the Marais criteria, classifying cases as definite, probable, or possible TBM. All patients underwent uniform clinical assessment, CSF analysis, neuroimaging, and immune marker testing (ALC and CD4 counts). CD4 counts were assessed via flow cytometry and ALC using automated analyzers. Despite differences in diagnostic certainty, the methods of assessment and management protocols were comparable across all three groups.

All patients with HIV coinfection, underlying malignancy, on immunosuppressive therapy, with underlying chronic kidney disease, Rheumatological, chronic liver disease, and age less than 18 years have been excluded from this study. Data were collected using an oral questionnaire regarding relevant history from the patient or caregiver, or by performing bedside clinical assessment.

Efforts to reduce bias

Efforts to reduce bias included standardized diagnostic criteria (MRC and Marais), uniform timing of immune marker assessments, prospective data collection, and objective outcome evaluation. Only HIV-negative patients were included to avoid confounding. However, being a single-center study, some selection bias may persist.

Statistical analysis

Using the single population proportion formula, the sample size was established. The p-value was set at 0.5, and the margin of error was set at 0.05 because no prior comparable research had been conducted in India. The non-response rate was set at 10%. We obtain a sample size of 53 after extrapolating the risk factor assessment studies to a design effect of 1.5.

For statistical analysis, data were entered into a Microsoft Excel spreadsheet and analyzed using SPSS (version 24.0; SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 5. We used descriptive statistics to analyze the baseline demographics.

Data are summarized as mean and standard deviation for numerical variables and counts and percentages for categorical variables. Qualitative (categorical) variables were expressed as proportions and percentages. The Chi-square test and Fisher's exact test were used to assess associations between categorical variables, as appropriate. Quantitative variables were expressed as mean \pm standard deviation (SD). The Student's *t*-test and one-way analysis of variance (ANOVA) were used to evaluate associations between quantitative variables.

Ethical considerations

The study protocol was reviewed and approved by the Institutional Ethics Committee of Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India.

Informed consent

Written informed consent was obtained from all participants before their enrolment in the study.

Results

During the study period, 65 patients who presented with a clinical suspicion of tuberculous meningitis (TBM) were screened. Following the initial assessment, 60 patients had their eligibility assessed. 53 of these patients were found to be eligible and satisfied the inclusion requirements. The study covered all 53 participants. Every one of them finished the nine-month follow-up and was incorporated into the final analysis. Following enrollment, no patients were excluded or lost to follow-up.

A total of 53 patients were enrolled, of whom 23 were males and 30 were females. Patients were categorized into Stage I, II, and III based on the Medical Research Council (MRC) grading system for tuberculous meningitis (TBM). Further classification was done according to the Marais criteria into possible, probable, and definite TBM.

Absolute lymphocyte count (ALC) and CD4 values were obtained at admission and after 9 months of treatment.

Table 1 illustrates the association between gender and outcome. Of the 53 patients enrolled, 23 were males and 30 were females. In the male group, 11 patients (47.8%) had a good outcome, while 12 patients (52.2%) had a poor

outcome. In the female group, 25 patients (83.3%) had a good outcome, and 5 patients (16.7%) had a poor outcome. The difference was statistically significant, with a p-value of 0.006 and a relative risk of 2.36, indicating that female patients were more likely to have a favorable outcome compared to males.

Table 1. Association of gender with outcome

OUTCOME	MALE	FEMALE	TOTAL	P VALUE	RELATIVE RISK
GOOD	11(47.8%)	25(83.3%)	36(67.9%)	0.006	2.36
POOR	12(52.2%)	5(16.7%)	17(32.1%)		
TOTAL	23	30	53		

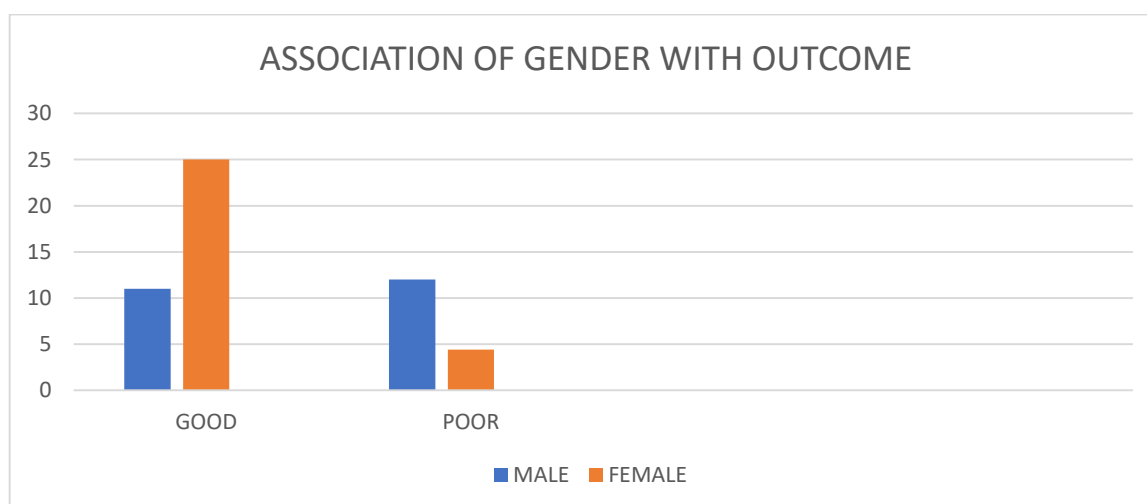


Figure 1. Association of gender with outcome

Table 2 illustrates the association between duration of symptoms and outcome. Of the 53 patients enrolled, 23 had a long duration of symptoms, and 30 had a short duration of symptoms. In the long-duration group, 14 patients (60.8%) had a good outcome, while 9 patients (39.2%) had a poor outcome. In the short-duration group,

22 patients (73.3%) had a good outcome, and 8 patients (26.7%) had a poor outcome. The p-value was 0.335 with a relative risk of 0.735, indicating that there was no statistically significant difference in outcomes between the two groups.

Table 2. Association of duration of symptoms with outcome

OUTOCOME	LONG DURATION	SHORT DURATION	TOTAL	P VALUE	RELATIVE RISK
GOOD	14(60.8%)	22(73.3%)	36(67.9%)	0.335	0.735
POOR	9(39.2%)	8(26.7%)	17(32.1%)		
TOTAL	23	30	53		

Table 3 illustrates the association between TBM stage and outcome. Of the 53 patients enrolled, 7 were in Stage 1, 33 were in Stage 2, and 13 were in Stage 3. Among the 7 patients in Stage 1, 6 (85.7%) had a good outcome and 1

(14.3%) had a poor outcome. In Stage 2, 26 patients (78.7%) had a good outcome, while 7 (21.3%) had a poor outcome. In Stage 3, only 4 patients (30.7%) had a good outcome, whereas 9 patients (69.3%) had a poor outcome.

The p-value was 0.004, indicating a statistically significant difference in outcomes across TBM stages.

Table 3. Association of TBM stage with outcome

OUTCOME	STAGE1	STAGE2	STAGE3	TOTAL	P VALUE
GOOD	6(85.7%)	26(78.7%)	4(30.7%)	36(67.9%)	0.004
POOR	1(14.3%)	7(21.3%)	9(69.3%)	17(32.1%)	
TOTAL	7	33	13	53	

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Table 4 illustrates the association between absolute lymphocyte count (ALC) and outcome. In this study, 53 patients were enrolled, of whom 40 patients were in the low ALC group and 13 patients were in the normal ALC group. Among the low ALC group, 26 patients (65%) had a good outcome, while 14 patients (35%) had a poor

outcome. In the normal ALC group, 10 patients (76.9%) had a good outcome, and 3 patients (23.1%) had a poor outcome. The p-value was 0.424 and the relative risk was 0.817, indicating that there was no statistically significant difference in outcome between the two groups.

Table 4. Association of absolute lymphocyte count with outcome

OUTCOME	LOW ALC	NORMAL ALC	TOTAL	P VALUE	RELATIVE RISK
GOOD	26(65%)	10(76.9%)	36(67.9%)	0.424	0.877
POOR	14(35%)	3(23.1%)	17(32.1%)		
TOTAL	40	13	53		

Table 5 illustrates the association between CD4 count and outcome. Of the 53 patients enrolled, 27 were in the low CD4 group, 7 in the normal CD4 group, and 19 in the very low CD4 group. Among the 27 patients in the low CD4 group, 19 (70.3%) had a good outcome, while 8 (29.7%) had a poor outcome. In the normal CD4 group, 5 patients

(71.4%) had a good outcome, and 2 (28.6%) had a poor outcome. Among the 19 patients in the very low CD4 group, 13 (68.4%) had a good outcome and 6 (31.6%) had a poor outcome. The p-value was 0.856, indicating no statistically significant difference in outcomes across the CD4 count categories.

Table 5. Association of CD4 count with outcome

OUTCOME	LOW CD4	NORMAL	VERY LOW CD4	TOTAL	P VALUE
GOOD	19(70.3%)	5(71.4%)	12(63.1%)	36(67.9%)	0.856
POOR	8(29.7%)	2(28.6%)	7(36.9%)	17(32.1%)	
TOTAL	27	7	19	53	

Table 6 illustrates the association between the duration of dexamethasone therapy and patient outcomes. Of the 53 patients enrolled, 33 received dexamethasone for less than 8 weeks, and 20 patients received it for more than 8 weeks. Among those treated for less than 8 weeks, 23 patients (69.6%) had a good outcome, while 10 patients (30.4%)

had a poor outcome. In the group treated for more than 8 weeks, 13 patients (65%) had a good outcome, and 7 patients (35%) had a poor outcome. The p-value was 0.723 and the relative risk was 1.09, indicating no statistically significant difference in outcomes based on the duration of dexamethasone therapy.

Table 6. Association of duration of dexamethasone with outcome

OUTCOME	< 8 WEEKS	>8 WEEKS	TOTAL	P VALUE	RELATIVE RISK
GOOD	23(69.6%)	13(65%)	36(67.9%)	0.723	1.09
POOR	10(30.4%)	7(35%)	17(32.1%)		
TOTAL	33	20	53		

Figure 2 illustrates the association between focal neurological deficits and clinical outcomes in TBM patients. Among the 53 patients enrolled, 23 presented with focal neurological deficits, while 30 had no such deficits. Of the 23 patients with focal deficits, 12 (52.1%) achieved a good outcome and 11 (47.9%) had a poor outcome. In contrast, among the 30 patients without focal

deficits, 24 (80%) had a good outcome and 6 (20%) had a poor outcome. This difference was statistically significant, with a p-value of 0.031 and a relative risk (RR) of 1.89, indicating that patients without focal neurological deficits were nearly twice as likely to experience favorable outcomes compared to those with deficits.

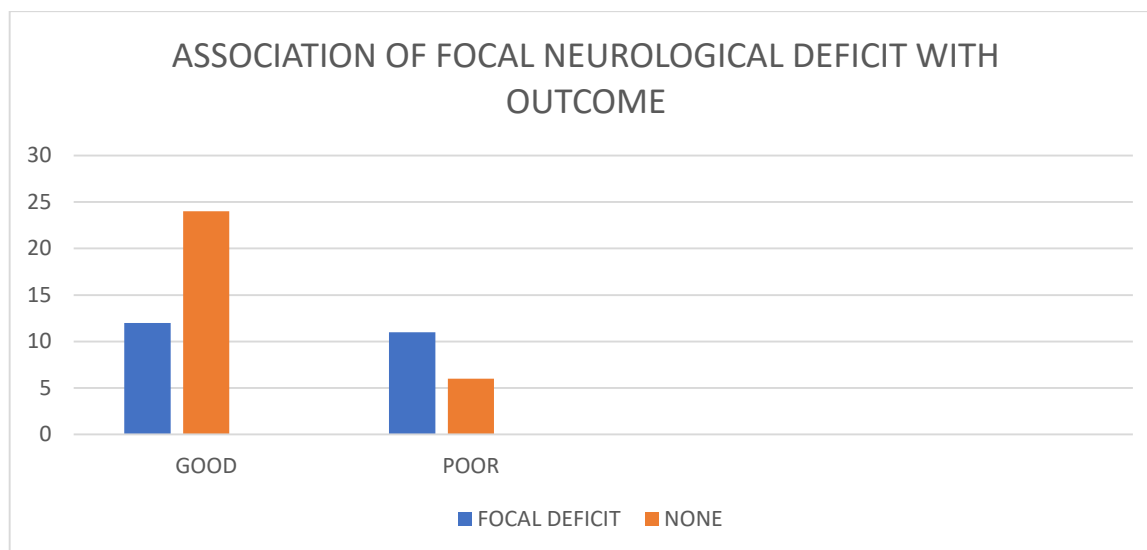


Figure 2. Association of focal neurological deficit with outcome

Discussion

Previous studies in the peripheral blood of TBM patients have demonstrated a marked decline in the absolute counts of lymphocyte subsets. These immune cells were shown to correlate, to some extent, with both humoral and cellular immunological responses, and their levels were directly associated with disease severity in a study by Mi et al [4].

In the present study, a statistically significant difference in outcomes was observed between male and female patients. Among females, 83.3% achieved a good outcome compared to only 47.8% of males. Conversely, 52.2% of males experienced a poor outcome versus just 16.7% of females. The observed difference was statistically significant ($p = 0.006$), and the calculated relative risk ($RR = 2.36$) suggests that female patients were more than twice as likely to achieve a favorable outcome compared to male patients.

These findings are consistent with previous literature reporting gender-based disparities in health outcomes across various clinical settings, as noted by J-Y Feng et al.

[5]. Several factors may contribute to this observed difference. Biological factors such as hormonal influences, variations in immune response, and genetic predispositions may underlie these sex-specific outcomes. Additionally, the poorer outcomes seen in males may be attributed to a higher prevalence of comorbidities, older age, and lifestyle factors such as smoking.

The study showed that 60.8% of patients with long symptom duration had a good outcome compared to 73.3% in the short-duration group. Conversely, poor outcomes were slightly more frequent in the long-duration group (39.2%) than in the short-duration group (26.7%). However, this difference did not reach statistical significance ($p = 0.335$), and the relative risk ($RR = 0.735$) suggests only a modest and non-significant trend favouring better outcomes in those with shorter symptom duration. These findings imply that while early presentation may be associated with better clinical outcomes, the duration of symptoms alone does not appear to be a strong or independent predictor of outcome in this study population. Previous studies in similar clinical contexts have suggested that delayed presentation



may contribute to worse outcomes due to progression of disease or missed therapeutic windows. The lack of statistical significance may be due to the limited sample size.

Among the 53 patients studied, 85.7% of those in Stage 1 achieved a good outcome, compared to 78.7% in Stage 2 and only 30.7% in Stage 3. Conversely, the proportion of poor outcomes increased with disease severity, from 14.3% in Stage 1 to 69.3% in Stage 3. This trend was statistically significant ($p = 0.004$), indicating that the stage of TBM at the time of diagnosis is a critical determinant of clinical outcome.

These findings are consistent with previous literature, Wang MG et al, which underscores the importance of early diagnosis and intervention in TBM. Advanced stages are typically associated with extensive neurological involvement, higher intracranial pressure, and systemic complications, all of which contribute to increased morbidity and mortality. The stark difference in outcomes between early and late stages reinforces the need for heightened clinical awareness and timely initiation of anti-tuberculous therapy [6].

The findings suggest that ALC/CD4 count at the time of TBM diagnosis is not significantly associated with clinical outcomes. While lymphopenia has been linked to impaired immune responses and adverse outcomes in various infections, including tuberculosis, the data did not demonstrate a statistically meaningful correlation. This may reflect the complex interplay of immune status, disease severity, and host response in TBM.

Although not statistically significant, the trend toward better outcomes in the normal ALC/CD4 group could imply a potential role of immune competence in influencing prognosis.

The study suggests that focal neurological deficits may serve as an early predictor of adverse outcomes in TBM. This was in concordance with previous studies. Renu Gupta et.al. Such deficits often reflect localised brain damage caused by cerebral infarcts, tuberculomas, or complications such as hydrocephalus or raised intracranial pressure. Their presence indicates more severe neurological involvement and may be associated with delayed diagnosis or inadequate early disease control. The nearly two-fold increased risk of poor outcome in patients with focal deficits reinforces the need for prompt and aggressive management. This includes early neuroimaging to identify structural lesions, timely initiation of anti-tubercular therapy, adjunctive corticosteroids, and consideration of neurosurgical intervention when appropriate. Furthermore, close neurological monitoring during hospitalization is crucial for optimizing outcomes [7].

Generalizability

Being a single-centre study, its results may not fully apply to other regions or healthcare settings.

Conclusion

In this study of HIV-negative TBM patients, we observed that lower TBM stage and female gender were significantly associated with better outcomes. Although absolute lymphocyte and CD4 counts did not show statistically significant associations with outcomes, trends favoring immune competence were noted. Further large-scale prospective studies are warranted to validate the prognostic role of ALC and CD4 counts in TBM management.

Limitations

A limited sample size and single-center design are two of the study's drawbacks that could restrict generalizability. Only two measurements of immune markers like CD4 and ALC were made, which limited the understanding of dynamic changes. We did not evaluate potential confounding variables like subclinical immunosuppression or dietary status. There was no advanced immune profile (e.g., CD8 numbers, CD4/CD8 ratio). Furthermore, since patients from tertiary care facilities sometimes reflect more severe conditions, selection bias can exist.

Recommendations

To confirm the predictive significance of ALC and CD4 counts in HIV-negative TBM, future research should involve larger, multicenter cohorts. To gain a better understanding of disease dynamics, enhanced profiling, including CD8 and CD4/CD8 ratios, and serial immune surveillance are advised. These indicators could help with treatment planning and risk assessment. To enhance patient outcomes, early diagnosis and intervention should also be prioritized.

List of abbreviations

ALC- Absolute Lymphocyte Count

CD4- Cluster of Differentiation 4

TB- Tuberculosis

TBM- Tuberculous Meningitis



HIV- Human Immunodeficiency Virus

MRC- Medical Research Council

GCS- Glasgow Coma Scale

ICU- Intensive Care Unit

OPD- Outpatient Department

Page | 8 Acknowledgement

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

All authors contributed to the study design, data collection, analysis, and manuscript preparation.

Data availability

The data generated during this study are available from the corresponding author upon reasonable request.

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